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14. ABSTRACT To assess, maintain, or improve a soldier's physical/physiological/psychological capability to function effectively under environmental and operational stress and to minimize adverse effects of stress on health safety and performance, the PBRC performs the following eight research tasks: 1) Clinical Laboratory for Human and Food Samples performs laboratory analysis of samples from studies conducted by the U.S. Army Research Institute of Environmental Medicine (USARIEM) and at PBRC in Tasks 4 and 8. 2) Stable Isotope Laboratory performs analyses to measure the energy expenditure and body composition of soldiers during prolonged field exercise and at PBRC in Tasks 4 and 8. 3) Stress, Nutrition and Mental Performance Laboratory continues multidisciplinary basic research studies of the interactions of stressors and nutrition on mental performance parameters in an animal model. 4) Stress, Nutrition and Work Performance uses human subjects to develop nutritional strategies to improve physical performance under intense physical stress. 5) Nutrient Database Integration Laboratory supports the Military Nutrition Division and PBRC research studies by providing dietary intake and analysis support. 6) Enhancing Military Diets targets health promotion through improved nutrition in basic combat training. 7) Stress Nutrition and Immune Function Laboratory has been inactive in this year (since 6/30/00). 8) Metabolic Unit Project allows new inpatient protocols to address specific issues in nutrition and metabolism that are important to the military mission.					
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Introduction

This is the final report for the specially funded cooperative agreement between the Pennington Biomedical Research Center (PBRC) and the U.S. Army Medical Research and Materiel Command (USAMRMC) of the Department of Defense (DoD). It covers the period 7/1/97 through 9/30/05 and reports on the activities of the project entitled, "Military Nutrition Research: Eight Tasks to Address Medical Factors Limiting Soldier Effectiveness."

The PBRC has a 17-year history of collaborative research with the Department of Defense (DoD). A series of specially funded cooperative agreements between the PBRC and the U.S. Army Medical Research and Materiel Command (USAMRMC) has provided high quality analytical laboratory, nutrition database, and metabolic unit support for DoD nutrition-related research programs. The program supports the RDT&E funded Military Nutrition Research Programs at the U.S. Army Soldier Systems Center (Natick, Massachusetts) and the U.S. Army Research Institute of Environmental Medicine (USARIEM) laboratories, as well as the Ration Sustainment Testing program. PBRC personnel have traveled to DoD field studies to collect samples, which are returned to the PBRC for laboratory analyses. Additionally, the PBRC has conducted research that complements and extends USARIEM's intramural program in areas of nutritional neuroscience, stress, physical, and mental performance, and garrison feeding. Though funded through earmarks, the PBRC program has been periodically successfully peer reviewed by an external panel from the Committee on Military Nutrition Research (CMNR), Institute of Medicine (1988, 1990, 1996, and 2002). This joint effort of PBRC and military researchers has led to significant improvements of operational rations, better understanding of warfighter energy and nutritional requirements, and modifications in garrison feeding.

The final report herein covers the third cooperative agreement series that began in 1988. The previous cooperative agreements are listed below.

Dates of Award	Title	Funding
7/1/97-9/30/05	Military Nutrition Research: Eight Tasks to Address Medical Factors Limiting Soldier Effectiveness	\$ 21,068,131
4/1/92-3/30/98	Military Nutrition Research: Six Tasks to Address Medical Factors Limiting Soldier Effectiveness	\$11,340,567
8/1/88-7/31/92	Effect of Food, Diet and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependents in a Peacetime Environment	\$3,845,000

In each of the three cooperative agreements, the CMNR of the National Academy of Sciences provided peer review prior to and during program implementation. Additionally, USARIEM approved all projects and provided consultation on research design for all projects that emanated from PBRC. Any modifications to the original research plan were approved by USARIEM prior to

implementation.

One of the advantages to this longstanding relationship is access by the DoD to the excellent facilities and equipment found at PBRC and described in detail in the Appendix.

The PBRC research projects aimed at military nutrition issues have been completed on or ahead of schedule with high quality control and are detailed extensively in quarterly, annual and final reports. The relationship has yielded a number of scientific publications as indicated later in this report.

The overarching goal of this research is to assess, maintain, or improve a soldier's physical/physiological/psychological capability to function effectively under environmental and operational stress and to minimize adverse effects of stress on health, safety and performance. To achieve that goal, over the eight year course of the project we have had three types of activities supported by the project, as outlined below:

1. Reference laboratory support for studies that emanated from Principal Investigators within the military:
 - Clinical Laboratory for Human Samples
 - Stable Isotope Laboratory
 - Nutrient Database Laboratory

We provide a listing of the research study titles that PBRC laboratories have supported under each of the above three Tasks, as "Key Research Accomplishments" of the individual tasks.

2. Use of specialized facilities at PBRC for clinical experiments:
 - Metabolic Unit

This Task has primarily served as a contractual "place-holder", making the excellent facilities at PBRC available should they be needed to serve DoD research interests. During the eight years of this work, no Metabolic Unit studies were performed.

3. Independent projects led by PBRC Principal Investigators:
 - Nutrition Neuroscience Basic Research Laboratory (1992-1997)
 - Stress, Nutrition and Immune Function (1998-2000)
 - Stress, Nutrition and Work Performance (1997-2003)
 - Stress, Nutrition and Mental Performance (1997-2003)

The types of activities detailed in (1) and (2), above, serve the primary function of supporting USARIEM investigations and they are ongoing. Some of the projects listed in (3) have evolved to other sources of funding. For example, the findings of the Nutritional Neuroscience Laboratory that acute restraint stress in rodents is associated with chronic alterations in body weight and food intake has led to continued investigations funded by the National Institutes of Health. Another example from that category has been the evolution of a weight management program that was developed under Task 6 to be applied to soldiers at Fort Bragg and to the Army's 94th Reserve Battalion. Those two projects, "Military Health Behaviors: Promotion of Healthy Weight and Fitness in Career Personnel, DAMD 17-03-2-0030" also called "Army H. E. A.L.T.H." and "Weight Measurements and Standards for Soldiers" are funded under separate Broad Agency Agreements (BAAs). These are described in the "Reportable Outcomes" section of Task 6 in this report.

Body

The project consists of eight tasks and is administered by the Principal Investigator, Donna H. Ryan, MD and her administrative assistant, Janice Warren. She has been assisted by the Co Principal Investigator named in 2005, Jennifer Rood, PhD. We describe background for each of the eight tasks, below.

1. Task 1. Clinical Laboratory for Human and Food Samples – Background:

The clinical laboratory provides support for military nutrition research by providing the following services:

- assistance with protocol development
- sample collection and processing on-site or in a field setting
- sample analysis
- new method development
- assistance with manuscript publication

The laboratory is accredited by the Health Care Financing Administration (HCFA) and the College of American Pathologists (CAP) and participates in the lipid standardization program of the Centers for Disease Control. Good Laboratory Practices guidelines are being followed in the laboratory. The Clinical Research Laboratory is staffed by licensed medical technologists, phlebotomists, and accessioners.

The laboratory is well-equipped for performing routine and specialized tests on clinical subjects. In 2004 over 250 different analytical procedures involving 200,000 tests were performed by the lab. The laboratory is comprised of five departments: chemistry, special chemistry, point of care testing, hematology, and urinalysis. Testing is performed on a variety of specimen types including blood, urine, sweat, saliva, and

feces. The Facilities Description in the Appendix provides a complete list of tests that are available in the Clinical Laboratory.

The laboratory was under the leadership of Dr. Richard Tulley until 2002 and since then it has been directed by Dr. Jennifer Rood.

Key Research Accomplishments:

During the grant period 1997-2005, the laboratory sent personnel to aid in specimen collection for 1) a field study in El Paso for the Sergeant Major's Academy study, 2) the Parris Island, SC Study wherein four trips were required, and 3) the Protein Supplementation Study in Quantico Virginia wherein three trips were required. In these circumstances, PBRC personnel operate under the direction of USARIEM personnel.

The laboratory supported studies which were performed at PBRC, specifically the exercise study that was conducted in-house as part of Task IV. The details of this support (and of all studies conducted in the field or at USARIEM) can be found in annual and quarterly reports.

The laboratory supported the following studies that were conducted in the field under the supervision of military Principal Investigators:

- Sergeant Major's Academy Study - El Paso
- Banderet Tyrosine Study
- Ranger 3 Study
- Assessment of Nutritional Status and Energy Expenditures and Determination of Gender Differences in Dietary Intakes of Combat Army Support Hospital (CASH) Personnel Subsisting on Meal-Focused Versions of the Meal, Ready to Eat
- Hot Weather Feeding Study
- Ranger 4 Study
- SFAS-6 Study
- Carbohydrate and Performance Study
- Post Exercise Nutrient Supplementation Study (PENS)
- Ranger Regiment Nutritional Survey
- Effects of Immune Egg Protein and Antioxidants on Muscle Soreness and Strength after Eccentric Exercise (Eccentric Exercise Study - EES) –Jennifer Jenner, PI
- Effects of Repeated Dosing of Caffeine on Vigilance, Cognitive and Physical Performance and a Sentry Duty Marksmanship Task (Caffeine and Sleep Deprivation – CSD) – Harris Lieberman, PI
- Assessment of Energy Expenditure and Nutritional Status of Female Navy Personnel Onboard Ship (Navy Study) – Beverly Patton PI
- Physical Fitness and Body Composition before and after a 2000 Km Unsupported Ski Trek across the Arctic Ocean (Arctic Norwegian Soldier Study)
- Longitudinal Analysis of Bone Density and Stress Fracture Rates in a Population of

West Point Graduates

- Assessment of Weight Status and Attrition of Female Marine Recruits during Recruit Training – Gaston Bathalon, PI (Parris Island, SC)
- Savannah – Ranger Study
- Pike's Peak Study – Ann Grediagin PI
- Virginia Supplement Study – Brad Nindl, Scott Montain, Co-PIs
- Growth Hormone 24 hour variation Study – Brad Nindl, PI
- Mood and Vigilance Following Quercetin Study – Craig Olson, PI
- Evaluation of Minimal or Non-Invasive Methods for Field Assessment of Nutritional and Metabolic Status – Mark Kellogg, PI
- Skidmore Study 2003-4 – Louis Marchitelli, PI
- Nitrogen Balance Study 2003-2005 – Matthew Pikosky PI
- Ho3-04 Study - Harris Lieberman PI

The details of these studies and the laboratory support for them can be found in the Quarterly and Annual Reports for this grant.

Conclusions:

The laboratory plays an important role in furthering the knowledge concerning nutrition in the military by providing routine and esoteric testing, custom method development, assistance with testing and collection protocols, field assistance with blood collection and processing, and collaboration on publications.

2. Task 2. Stable Isotope Laboratory - Background:

The research conducted by the Stable Isotope Laboratory is in the area of energy and water requirements, and changes in body water, of soldiers, often under harsh environmental conditions. The method used to determine energy requirements is the doubly labeled water (DLW) technique, which involves oral administration of water labeled with the stable isotopes, ^2H and ^{18}O . Saliva and urine samples are then obtained for periods of four to 14 days, longer with redosing. Water intake can be determined using only the ^2H labeled water. The use of doubly labeled water for measurement of energy expenditure was developed as a field technique for use in small animals. The method is based on the premise that after a loading dose of $^2\text{H}_2^{18}\text{O}$, ^{18}O is eliminated as CO_2 and water, while deuterium is eliminated from the body as water. The rate of CO_2 production, and, hence, energy expenditure, is calculated from the difference of the two elimination rates. The only requirement of subjects is to give urine and saliva specimens before and after drinking an initial dose of $^2\text{H}_2^{18}\text{O}$, and then return in one to two weeks to give a final urine specimen. During the period between the two urine and saliva samplings, subjects are free to carry out their normal activities and are not required to maintain extensive diaries. The doubly labeled water method has been extensively validated in humans under controlled settings, but there are confounding factors that need to be considered in field studies, particularly in Army Field Studies. Among these are changes in location or food and water supply

immediately preceding, or during an energy expenditure study. These changes may cause a change in baseline isotope abundance and, therefore, interfere with the accuracy of the energy expenditure measurement. This has occurred in a previous field training exercise involving the study of the MRE and RLW rations. This is a particular problem with studies such as the Ranger Training Studies, in which soldiers are moved to different parts of the country during the study. Therefore, a group not receiving labeled water must be followed to make any corrections in baseline isotope shifts.

Hydration status is another main focus for some Army studies. Using the cheaper and more readily available deuterium tracer, either changes in total body water can be followed during a study, or water turnover (intake) can be measured.

One advantage of the DLW method is that it uses stable isotopes so there is no radiation exposure.

The PBRC Stable Isotope lab has provided analytical tech support, isotopically labeled infusate and professional consultation and expertise in interpreting the analytical data. Jennifer Rood, PhD (current head and permanent Associate Director of the Stable Isotope Lab) oversees a team of technical staff and collaborates with Andy Young (Chief, Military Nutrition Research Division at USARIEM) regarding the design and execution of doubly labeled water studies, as well as studies of protein turnover and gluconeogenesis using stable isotope tracer technology. Until June, 2005, the laboratory was under the direction of Jim DeLany, PhD and his assistant, Lauri Byerley, PhD.

The lab determined the appropriate methods to quantitate glucose appearance, disappearance ($6,6\text{ d}_2$ glucose) and gluconeogenesis ($2\text{-}^{13}\text{C}$ glycerol). We also identified appropriate tracers to quantitate protein synthesis (^{15}N phenylalanine, 2,3,5,6 D_4 tyrosine, and ^{15}N tyrosine). The lab developed an Excel spreadsheet that quickly calculates each subject's isotope bolus and isotope continuous infusion based on their weight. USARIEM sends the subjects weight several days prior to the study. We calculate the bolus and continuous infusion dose and send this information back to USARIEM and Tufts. At Tufts, the nurses check the calculations before the solutions are formulated by the pharmacist at Tufts. A great deal of effort was spent determining the appropriate technique for manufacturing the isotope bolus and continuous infusion.

The pharmacist who prepared the solutions was concerned with solubility, concentration, storage life, and sterility and pyrogenicity. Tyrosine is extremely insoluble. We conducted a literature search and contacted another research group for a formula to solubilize the tyrosine. Once we had this information, the pharmacist prepared the first stock solutions of the isotopes.

PBRC's Stable Isotope Core developed the GC/MS methods for analyzing these samples. We worked out all the problems (due to inaccurate published methods) in the generation of the saccharic acid derivatives for calculation of gluconeogenesis.

Glucose, glycerol, amino acids and saccharic acid have all been purified, derivatized and analyzed on the mass spec for subjects 103 (periods 1-3) and 104 (periods 1-3). Data from those analyses is currently being processed.

Deleted: Amino acid fractions were collected from the sample purifications but it appears that the recoveries were too low for accurate determinations of phenylalanine and tyrosine.

Key Research Accomplishments:

During the grant period 1997-2005, this task supported the following studies:

- 75th Ranger Regiment Study – Savannah
- Ranger 4 Study
- Norwegian Rangers Study 97
- Norwegian Rangers Study 98
- Effects on Energy Expenditure, Water Turnover and Hydration Status During a 63 Day Marine Field Exercise
- Infantry Office Training Course, Quantico, summer 1999
- Infantry Office Training Course, Quantico, winter 1999
- Simulation of a Disabled Submarine (DISSUB) with Eight Volunteer “Survivors” 2000
- Shipboard study of energy expenditure for men and women (200-2001)
- Ft. Carson study of energy balance among members of the 10th Special Forces Group (2000)
- Sustained Operations Study (SUSOPs) of cognitive and physiological changes (2000-2001)
- Warfighter Physiological Status Monitoring (WPSM): Thermal Status and Water Intake during a Warm Weather US Marine Corps Marksmanship Training Course Study
- Assessment of Weight Status and Attrition of Female Marine Recruits During Basic Training 2001-2002, Parris Island
- Warfighter Physiological Status Monitoring (WPSM) Joint Field Study with the 1st Battalion (ABN), 509th Infantry OPFOR, Fort Polk 2002
- Pike’s Peak Study of antioxidant supplementation and carbohydrate supplementation on acute mountain sickness and other endpoints – USARIEM Altitude Group
- Nitrogen Balance Study 2002-3
- Protein Turnover and Gluconeogenesis 2002-2005, Andy Young PI

The details of these studies and the laboratory support for them can be found in the Quarterly and Annual Reports for this grant.

Reportable Outcomes:

- Dr. DeLany obtained the following grant as a result of this work: Defense Women's Health UIS #DE950237. Determination of daily energy requirements and activity patterns of servicewomen. James P. DeLany (Principal Investigator), \$819,093, 10/1/96 - 9/30/01.

Conclusions:

The laboratory plays an important role in furthering the knowledge concerning nutrition in the military by providing for analysis of water turnover, energy expenditure in the field, and glucose appearance, disappearance and gluconeogenesis. These are highly specialized techniques and are not available in commercial laboratories. The value of these techniques is illustrated by the results of several of the studies detailed below.

- Average total daily energy expenditure during the Marine construction mission was 3330 kcal/d. Construction engineers expended more energy than administrative and support personnel. Current ration policy (NSOR) provides sufficient energy to meet the demands of combat engineers performing this type of mission. Negative energy balances were greatest for construction engineers consuming T Rations. As time progressed these negative energy balances increased. While the Marine leadership continually stressed the importance of fluid consumption, the difficulty of individuals obtaining and consuming sufficient fluids while away from base camp was a problem. The education of every soldier and marine on the importance of fluid consumption and the consequences of hypohydration needs to continue.
- The energy expenditures reported for the Norwegian Ranger Training studies are some of the highest reported energy expenditures we have observed in military personnel 6250 ± 770 kcal/d this year, 500 kcal/d higher than was observed in the Norway '97 study of 5650 ± 800 kcal/d. The only energy expenditures approaching this level were those observed in the Marine Crucible event and the "Mountain Class" phase of the Ranger Training studies.
- The average energy expenditure during the Winter Infantry Officer Training Course was quite high, 6472 ± 816 kcal/d. We expected somewhat elevated energy expenditure, as the weather was cold, soldiers were carrying around 100-150 lbs. and were getting very little sleep. Average energy expenditure during the summer study under otherwise similar conditions was also high, but was considerably lower than during the winter (4107 ± 600 kcal/d).
- The participants in the simulated disabled submarine survival study had a surprisingly high-energy expenditure, considering their low activity level. The exposure to hypercapnic, mildly hypoxic cold conditions appear to cause a significant increase in energy expenditure. The fluid balance of the subjects during this study did not appear to be dramatically affected over the course of the study, as the total body water lost by the end of the study was only 0.4 kg.
- The average daily energy expenditure of the female shipboard subjects was 2808 ± 429 kcal/day. This is significantly less than the energy expenditure of the male subjects. However, this difference in daily caloric energy expenditure can be explained by a difference in fat free mass. The men had a significantly greater fat free mass than the women.
- Special Forces Qualified soldiers were shown to have a high energy expenditure of 4100 kcal/d, with a subsequent energy deficit of 1100 kcal/d.

3. Task 3. Stress, Nutrition and Mental Performance - Background:

This laboratory was under the direction of Ruth Harris, PhD, from the inception of the grant until January 1, 2001, when Roy Martin, PhD became the leader of the Task. Dr. Martin continues to lead the Task until funding cuts required that the Task be inactivated in spring 2003. Dr Harris relocated to the University of Georgia in 2000 and was recently promoted to Professor in the Department of Foods and Nutrition. Dr. Martin remains on the faculty at PBRC and is jointly appointed at LSU A&M.

The overall goal of the Task under Dr. Harris's leadership was to use a multidisciplinary team to identify nutritional interventions that have a positive impact on stress-induced behavior. Sleep deprivation and restraint are the models used by the laboratory to identify nutritional strategies that influence learning behavior, memory and anxiety. Under Dr. Martin, the multidisciplinary team approach continued with a focus on regulation of food intake, the chronic effects of repeated acute stress on body weight regulation and identification of genetic markers for stress responsiveness. A particular interest was in activity of the melanocortin system as a genetic marker for stress responsiveness.

Key Research Accomplishments:

The following projects were undertaken during this grant from 1997-2003 and the following list details the team members who spearheaded the work:

- Operant Behavior in Stressed Rats Supplemented with Tyrosine – Bradley Youngblood
- CRF Receptors, Stress and Behavior – Gennady Smagin
- Measurement of Tissue Urocortin mRNA by Ribonuclease Protection Assay and Characterization of an anti-urocortin (UCN) antibody – Xiaolang Yan, You Zhou and Gennady Smagin
- Apolipoprotein E: A Potential Candidate as Genetic Marker for Stress Susceptibility – You Zhou, David Elkins and Leigh Anne Howell
- Glucose Utilization in Rats Exposed to Restraint Stress – Jun Zhou, Xiaolang Yan and Ruth Harris
- Involvement of Central CRF Receptors in the Regulation of Food Intake and Body Weight of Rats Subjected to Repeated Restraint Stress – Gennady Smagin, Ruth Harris, Leigh Anne Howell
- The Effect of Two Bouts of Repeated Restraint on Body Weight, Body Temperature, and Serum Cytokine Concentrations – Ruth Harris
- The Effect of Dietary Amino Acids on Spatial Memory in Rats Sleep deprived by the Flower-Pot Technique – Bradley Youngblood, David Elkins, Gennady Smagin, and Ruth Harris
- The Effect of Repeated Restraint Stress on Post-Stress Energy Metabolism - Jun Zhou, Xiaolang Yan, Leigh Anne Howell, Jacob Simpson, Tiffany Mitchell, Bradley Youngblood, Ruth B.S. Harris

- The Role Of Leptin In The Response To Repeated Restraint - Arica Guthrie, Tiffany Mitchell, Jacob Simpson, Ruth Harris
- The Effects of Rapid Eye Movement Sleep Rebound on Spatial Learning and Physiology of Rats – Bradley D. Youngblood and Ruth B. S. Harris
- Involvement of Urocortin in the Response to Stress – Gennady Smagin, Xiaolang Yan, Leigh Anne Howell and Mingxia Shi
- The Effect of Restraint Stress on Brain Glucose and Palmitate Utilization – Ruth Harris, Bradley Youngblood, Jun Zhou, Gennady Smagin
- Genetic Markers for Stress Responsiveness: the Effect of Restraint on Mice Overexpressing Agouti Protein – Ruth Harris, Tiffany Mitchell, Jacob Simpson, Jun Zhou and Mingxia Shi
- Identification of Natural Urocortin Antisense RNA in Rat Tissues – Mingxia Shi
- Changes in Fat Glucose Transport and Beta Adrenergic Receptor During The Post Stress Period in Rats Exposed to Repeated Restraint Stress – Jun Zhou, Mingxia Shi, Sonja Thomas, Tiffany Mitchell, Ruth Harris.
- The Effect of Food Availability and Timing of Stress on Weight Loss in Rats – Ruth Harris, Tiffany Mitchell, Sadie Herbert and Jun Zhou.
- Protective Effect of Insulin on Dopaminergic Neurons – Patrick Awenowicz and Xiaochun Xi
- The Melanocortin System as a Marker for Stress Responsiveness – Jun Zhou, and Mingxia Shi
- The Effect of Leptin on the Inflammatory Response to Endotoxin in db/+ and db/db Mice – Ruth Harris, Mary Boudreau, Dianne Dunning, Tiffany Mitchell
- The Effect of Endotoxic Stress on Adipose Tissue Metabolism in Rats – Jun Zhou, Roy Martin, Ruth Harris
- Stress Induced Cytokine Signaling – Mary Boudreau
- Stress, Nutrition and Immune Function – Mary Boudreau and Lisa Ballard
- Agouti protein as a potential maker for stress-responsiveness – Jun Zhou, Roy Martin
- Development of the model of repeated restraint stress as a potential model of Post-Traumatic Stress Disorder – Roy Martin
- Hunger, Satiety and Food Reward – Roy Martin, Colby Danna and Bing Li
- The Effect of Repeated Restraint Stress on Subsequent Patterns of Hormone Release in Rats – Haiyan Gu, Tiffany Mitchell and Ruth Harris
- The Effect of a Fish Oil Diet on the Response to Repeated Restraint in Rats – Emelia Papkonstantinou
- The Response to a Second Stressor in Rats that have been exposed to repeated Restraint Stress – Mariano Russo, Tiffany Mitchell and Ruth Harris
- Norepinephrine Inhibits Rat Preadipocyte Proliferation – Dorothy Hausman, Jie Lu
- Dose Response for CRF Sensitivity – Emilia Papkonstantinou and Tiffany Mitchell
- The Role of CRF2 Receptors in the Energy Balance to Response to Acute Stress – Haiyan Gu, Tiffany Mitchell and Ruth Harris

- NPY mRNA expression in hungry rats and satiated rats fed a palatable diet. Jun Zhou, Xiaochun Xi, David Roane, and Roy Martin.
- Probe labeling with fluorescence and hybridization using total RNA for microarray. Jun Zhou and Xiaochun Xi.
- Modification of the method of fluorescent in situ hybridization using blocking reagent. Xiaochun Xi, Bing Li and Roy Martin.
- Modification of fluorescent in situ hybridization by using three antibodies. Xiaochun Xi, Jun Zhou and Roy Martin.
- Expression of Glut-2 and glucokinase protein in Zucker rat pancreas. Bing Li, Xiaochun Xi, Roy J. Martin.
- Probes and primers design for real-time PCR. Xiaochun Xi, Jun Zhou and Roy Martin.
- The effects of low versus high glycemic index starch on weight gain and fat accumulation in rats. Maren Hegsted, Michael Keenan, Carol O'Neil, Anne Francis and Kathleen McCutcheon.
- Equivalent food intake in 2-DG injected rats and food restricted rats. Samuel Colby Danna, Jun Zhou, David Roane, and Roy Martin.
- Methods employed for the cannulation of the 3rd cerebral ventricle. Samuel Colby Danna.

The details of these studies and their results can be found in the Annual and Quarterly reports that were submitted for this grant.

Reportable Outcomes:

- Jun Zhou completed requirements for her Ph.D. in Veterinary Sciences at Louisiana State University. Her graduate research project was supported by this grant and her thesis title was "The effect of repeated restraint stress on peripheral energy utilization in rats." Her diploma was awarded in August, 1999.
- You Zhou, Ph.D. left the group in July 1998 to take a Research Assistant Professor position at University of Nebraska Lincoln. His responsibilities include Manager of the Microscopy Core Research Facility and Director of the Antibody Core Facility.
- Gennady Smagin, Ph.D. left the group in 1999 to take a faculty position at Louisiana State University Medical Center in Shreveport. This was a joint appointment at the Departments of Psychiatry and Pharmacology.
- Arica Guthrie, a high school student from Washington, DC, spent eight weeks in the laboratory during the summer of 1998 as a participant in the NASA SHARP PLUS program sponsored through Southern University, Baton Rouge. She conducted the leptin dose response study that is described in the body of this report.
- Emilia Papkonstantinou was a graduate (PhD) student supported with this grant. She received the 2001-2 Holly B. Alley Scholarship from the Georgia Nutrition Council, the 2002-3 June and Bill Flatt Nutrition Excellence Graduate Scholarship, the 2002 Georgia Nutrition Conference student Presentation Award

and was inducted into the Blue Key Honor Fraternity. She is now a faculty member of the Nutrition Department in Athens, Greece.

- Dr. Ruth Harris submitted an RO1 for support of the work which emanated from this award. The title of her award is, "Chronic effects of acute stress" and the funding period is from March 2004 to February 2009.
- Dr. Bing Li completed the requirements for a PhD in May 2005. Her Dissertation was entitled "Glucose Transporter 2's role in brain glucose sensing" and was converted into two manuscripts. She is currently in a Postdoctoral position at the Ohio State University.
- Dr. Jun Zhou was awarded a Biotechnology Education for Students and Teachers (BEST) postdoctoral fellow. In addition to conducting research on dietary resistant starch and nutrient signaling in the gut, she has trained teachers and students in the summer program and has give lectures to the participants of the BEST program. Jun was also awarded a pilot project grant which allowed her to collect enough preliminary data to support grant submissions to USDA and NIH.
- Dr. David Roane completed his sabbatical in Martin's laboratory in 2004. He has returned to the University of Louisiana at Monroe and was appointed head of the department of Biological Sciences. He was recently awarded an NIH grant.
- Dana Colby was admitted to the LSU Medical School. While working in the lab, Dana discovered that low doses of a beta cell toxin injected into the brain selectively destroy GLUT-2 expressing cells which subsequently impaired centrally-mediated, glucoprivic feeding.

Conclusions:

The results from our studies investigating behavior and physiology in rats during the recovery period following acute or chronic stress provides some of the first evidence that there are long-lasting effect of stress on cognitive and metabolic functions. Understanding these responses will facilitate development of protocols for promoting rapid recovery from stressful conditions. Identification of factors influencing UCN expression provides evidence for a role for UCN in the stress response and further studies are needed to clarify which biological responses to stress, all of which previously have been attributed to CRF, may be mediated by UCN. The expression of a naturally occurring antisense mRNA transcript for urocortin may represent a mechanism by which stress-induced urocortin expression can be regulated in a site-specific manner. This would allow specificity of behavioral and physiological responses to stress. The preliminary studies examining the effects of stress on brain glucose metabolism were intended to parallel human studies carried out at Natick in order to provide an animal model for testing the optimal time and composition of nutritional supplements that improve physical and cognitive function during extreme physical stress. Continuing investigation of proteins that act as peripheral markers for stress responsiveness may ultimately lead to a routine screen that will predict the potential for cognitive or physical performance of an individual in stressful environments.

The results from our studies investigating behavior and physiology in rats during the recovery period following acute or chronic stress provides some of the first evidence that there are long-lasting effect of stress on cognitive and metabolic functions. Understanding these responses will facilitate development of protocols for promoting rapid recovery from stressful conditions. Demonstration of changes in α -adrenergic receptor number in adipose tissue 24 hours after the end of stress is one of the first observations describing a sustained physiological response during the post-stress period. It also helps to explain the changes in body composition that occur in the days immediately following the end of exposure to stress. In addition, repeated restraint stress provides a unique model for investigating mechanisms that normally control body weight and body composition and how these regulatory systems are chronically disrupted by acute stress.

Demonstration of the involvement of the melanocortin system in determination of the responsiveness to stress suggests that it may be used as a marker for stress responsiveness. Indications that animals in which the system is disrupted due to a genetic manipulation have an altered sensitivity to stress, whereas acute manipulation of the system does not change stress responsiveness, strengthens the argument for this system being used as a genetic marker for stress responsiveness. Evidence that mice that over-express agouti protein are more responsive to stress than their wild-type controls provides the first evidence for a new marker and also implicates the melanocortin system in the behavioral and physiological response to stress. Continuing investigation of proteins that act as peripheral markers for stress responsiveness may ultimately lead to a routine screen that will predict the potential for cognitive or physical performance of an individual in stressful environments.

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Food reward and the overriding of satiety signals lead to obesity. The mechanisms appear to involve the dopamine system and specifically the dopamine transporter as a marker of dopamine stimulation. The palatability of food in this particular experiment is defined as the preference and amount of the food consumed. These basic food reward studies are aimed at identifying genes that control over-consumption of palatable foods.

Despite a highly productive publication record, this task was discontinued in 2003 because of funding difficulties.

4. Task 4. Stress, Nutrition and Work Performance - Background:

This task was under the leadership of Jeff Zachwieja, PhD from the inception of the grant until 2000. The project was taken over by a new leader Eric Ravussin, PhD in June, 2000 and continued until funding cuts required that it be inactivated in January,

2003.

Under Dr. Zachwieja's leadership, this task designed a series of studies to address issues of relevance to the military. The first experiments addressed protein requirements in conditions of physical stress. Because during periods of field missions, soldiers are self selecting a diet deficient in carbohydrate (and energy) but maintain protein intake, it is of interest to know whether 1) an increased protein content in the rations provided stimulates an increase in total energy intake, and 2) high levels of protein or amino acid intake help to maintain or improve physical performance under conditions of energy deficiency. Another experiment tested the hypothesis that branched-chain amino acids consumption would delay the exercise time to fatigue.

Following the assumption of Task leadership by Dr. Eric Ravussin (June, 2000), this task was refocused to address two major research questions: a) continuation of studies of nutritional approaches to enhance performance, and b) initiation of new studies designed to better understand the factors involved in the etiology of weight gain and obesity. The increasing number of Army personnel not meeting body weight standards and the shrinking pool of adolescents to recruit from (lower natality in the 80s and increased prevalence of obesity) is of concern to the Department of Defense. The focus of this Task is to better understand the factors leading to weight gain in adults and to prevent obesity at a younger age, with an emphasis on studies designed to improve our understanding of the interaction between environmental factors (such as nutrition and physical activity) and the genetic predisposition for weight gain.

To address these issues, this Task performed the accomplishments listed below.

Key Research Accomplishments under Dr. Zachwieja:

- Developed a laboratory model in humans to study the effects of moderate energy restriction on physical performance of healthy young adults has been developed.
- Conducted a study at PBRC in 1998-1999 involving 13 men and 11 women. The details of the study and its results can be found in a publication (Zachwieja JJ, Ezell DM, Cline AD, Ricketts JC, Vicknair PC, Schorle SM, Ryan DH. Short-term dietary energy restriction reduces lean body mass but not performance in physically active men and women. International Journal of Sports Medicine, 22:310-316, 2001).
 - Demonstrated that moderate (750 kcal/day), short-term (14 days) energy restriction in physically active young men and women results in weight loss. A majority of this weight loss comes from the lean body mass compartment.
 - Demonstrated that dietary protein intake of 1.3 grams/kg does not appear adequate to prevent body nitrogen loss during short-term energy restriction in physically active young men and women.

- Demonstrated that moderate, short-term energy restriction in physically active young men and women does not impair indexes of physical performance.
- Conducted a study in eight male subjects who were highly motivated endurance cyclists.
 - Demonstrated that addition of branched chain amino acids to a carbohydrate drink does not interfere with the ability of a carbohydrate drink to enhance exercise performance. In fact, in some individuals ingestion of a carbohydrate drink containing branched chain amino acids may be more effective at delaying fatigue than carbohydrate alone.
 - Branched chain amino acid ingestion seems to reduce prolactin secretion during exercise. This is suggestive of reduced serotonergic activity indicating that if central fatigue (emanating from serotonergic systems) plays a significant role in the overall development of fatigue during prolonged exercise, then branched chain amino acid ingestion may be one way to temper this component.

Key Research Accomplishments under Dr. Ravussin:

The research projects were developed under Dr. Ravussin's leadership are listed as follows:

- Metabolic Understanding of Energy Balance. Lilian de Jonge and Eric Ravussin
- Energy Metabolism in Response to 72 Hours Overfeeding and Fasting in Obesity-prone and Obesity-resistant Individuals. Submitted to HSRRB Log #A-10191– Enette Larson-Meyer and Eric Ravussin
 - Influence of Dietary Fat on Training and Performance
 - Influence of Diet on Recovery of Intramuscular Substrates and Subsequent Performance in Endurance Trained Men and Women.
- Prevention of Obesity – Melinda Sothorn and Eric Ravussin
 - A 6-month Randomized and Controlled Trial to Determine the Feasibility of an Efficacy of and After-School Obesity Prevention Program in 13-15 Year Olds
 - A School/Community Based Intervention to Prevent Adult Obesity in Overweight Adolescents: a Three-year Randomized and Controlled Trial
- Functional Genomics of Energy Balance and Training – George Argyropoulos and Eric Ravussin
 - -Identification and characterization of the promoter of *hAGRP*
 - -Identification, functional characterization of polymorphism in promoter of *hAGRP*
 - -Association of polymorphism in promoter of *hAGRP* with obesity and type 2 diabetes in Africans
 - -Identification of a common polymorphism in the promoter of the *Resistin* gene
 - -Functional characterization of the polymorphism in the promoter of the

Reportable Outcomes:

- Julie Ricketts received a Masters degree while being supported on this award.
- Paul Moran received a Masters degree while being supported on this award.
- Eric Ravussin submitted a grant application to the NIH titled "Metabolic Adaptations to Two-Year Caloric Restriction." It is funded as UO1 AG020478, from 03/15/2002 – 02/28/2009.
- Eric Ravussin submitted a grant application to the NIH titled "Fat Cell Size, Muscle Lipid Infiltration and Insulin Resistance", 5 RO1 DK060412 and it was funded from 09/30/2001 – 07/31/2006.
- George Argyropoulos submitted a grant application to the NIH titled, "Agouti Related Protein and its Role in Human Obesity" to extend the studies undertaken in this grant. The award was an RO1 and was funded from 3/01/2003 to 2/29/2008.
- Enette Larson-Meyer submitted a grant application to the NIH titled, "Impact of Diet on Intramyocellular Lipid, Endurance Performance and Insulin Sensitivity", Career Development Award. The award, K01 DK062018-01, was funded from 05/27/2000 to 06/30/2005.
- Melinda Sothorn submitted a grant application to the NIH titled, "Insulin Sensitivity in Children with Low Birth Weight. The award, R01 HD041071, was funded from 09/24/2004 to 08/31/2006.
- Melinda Sothorn submitted a grant application to the NIH titled, "Mechanisms for the Metabolic Syndrome in Prepubertal African American and Caucasian Youth." It was funded from 05/16/2005 to 02/28/2010.
- Dr. Melinda Sothorn relocated in 2004 from Baton Rouge to New Orleans to become an Associate Professor at the Louisiana State University Health Sciences Center in New Orleans as Director, Section of Health Promotion in the School of Public Health.
- Dr. Enette Larson-Meyer left Pennington in 2003 to become an Assistant Professor of Human Nutrition, Department of Family & Consumer Sciences at the University of Wyoming, Laramie.
- The Journal of Applied Physiology reported that the paper, Argyropoulos, G Harper ME. Molecular Biology of Thermoregulation Invited Review: Uncoupling proteins and thermoregulation, J Appl Physiol 92: 2187-2198, 2002 was among the top 10 most read papers of that year.

Conclusions:

Fourteen days of moderate energy restriction (750 kcal/day) in physically active young men and women results in body weight loss. A majority of this weight loss comes from the lean body mass compartment. Despite reductions in lean body mass, performance of specific exercise tasks which depend on muscular strength, power and endurance is not impaired.

Several subjects complained of muscle soreness and fatigue near the end of the first week of training (baseline period). This was likely the result of an increase in frequency (i.e., daily exercise training) of exercise training. Unfortunately, we made our baseline measurements of physical performance near the end of the first week. However we felt it was important to make the baseline measurements at this time because we wanted all subjects to be participating in a similar level (and frequency) of training when such measurements were made. Nonetheless, we may not have obtained a "true" baseline measurement of physical performance because of the existing level of fatigue. To better interpret the physical performance results, we will conduct another experiment in which measures of performance variables are made before and 7-10 days after increased frequency (daily) in exercise training.

Results from the branched chain amino acid study, completed within the past budgetary year, suggest that ingestion of a carbohydrate drink containing branched chain amino acids may be as useful, and in some individuals more useful than carbohydrate ingestion alone at delaying fatigue during prolonged physical work. Further, it was shown that branched chain amino acid ingestion decreased prolactin levels during exercise. Prolactin secretion is a reasonable indicator of activation of serotonergic systems. Thus, if serotonergic activation of central fatigue plays a significant role in the development of overall fatigue during prolonged physical work, then branched chain amino acid ingestion may be one way to offset this component and in doing so improve physical performance. This study adds to a significant body of research evaluating the efficacy of branched chain amino ingestion for the purpose of improving exercise performance. Future studies should focus on between subject variability in prolactin response to prolonged exercise and determine if individuals with an exaggerated prolactin response respond more positively to branched chain amino acid ingestion.

Our work with the AGRP and Resistin genes is expected to elucidate the genetic pathways that regulate food intake and the utilization of energy stores in humans. This research provides insight into the physiologic mechanisms by which the body regulates weight and into an understanding of how some individuals may be genetically predisposed to weight gain or to resist weight gain. Advancing scientific knowledge in this area is critical to the military interest, since overweight impacts performance negatively. Furthermore, overweight is a prevalent military condition, since failure to meet weight standards currently occurs in ~5% of career military personnel each year.

While we were unable to complete any experiments after 2001, due to extremely slow review times from the HSRRB, we do have the protocols on file and we were able to pursue other sources of funding for many of these ideas. Due to funding difficulties in 2003, this Task was discontinued.

5. Task 5. Nutrient Database Integration Laboratory – Background:

Assessing dietary intake is essential in determining the soldier's nutritional needs

and how those needs interface with other aspects of military performance. PBRC currently participates in field studies planned and conducted by the Military Nutrition Division of USARIEM by providing assistance with and analysis of dietary intakes collected during military field studies. That participation includes the following:

- Support for USARIEM field studies requiring data collection and data entry needs
- Support for PBRC in-house Military Nutrition Tasks
- Continued programming efforts directed toward meeting computer needs of both USARIEM and PBRC Military Nutrition Tasks

The Nutrient Database Integration Laboratory provides essential services for military operations. This Task oversees the operation of MiDAS, the database containing nutrient information for all operational rations, in addition to the USDA Standard Reference Foods and the USDA Food Survey Database food files. The Task provides critical support to studies which seek to improve soldier nutrition in a variety of field settings.

The Task Leader, Dr. Catherine Champagne and her staff are proficient in all aspects of nutritional intake assessment, including the Food Frequency Questionnaire, Food Diary Analysis, and Dietary Recall. The Leader and staff have been trained in the USDA multiple pass methodology. The Task Leader is a trained food scientist and has unsurpassed knowledge of nutrient database operations.

Key Research Accomplishments:

- This Task oversees the operation of MiDAS, the Army's database containing nutrient information for all operational rations, in addition to the USDA Standard Reference Foods and the USDA Food Survey Database food files. This Task developed special software to facilitate collection of dietary data at remote locations.

This task supported the following studies that were initiated by USARIEM investigators.

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- Nutritional Assessment and Dietary Education of U.S. Army Sergeants Major Academy Students in Garrison. A Three Phase Study conducted in September, 1996; December, 1996, and March 1997. Biggs Army Airfield, Fort Bliss, El Paso, TX
 - Day-to-day Variation in Fat Intake: Establishing Normative Data to Identify Potential Risk for Weight Gain
 - Diet and Coronary Heart Disease Risk Factors in Students at the US Army Sergeants Major Academy.
- Assessment of Nutritional Status and Energy Expenditure and Determination of Gender Differences in Dietary Intakes of Combat Service Support Personnel

Subsisting on Meal-Focused Versions of the Meal, Ready to Eat, 29 April 1997 to 13 May 1997. Camp McCall, North Carolina.

- Impact of Creatine Intake on Physical Performance, Fort Bragg, North Carolina. 28 August 1997 through 28 September 1997
- The Effects of Carbohydrate Supplementation on the Performance of Combat Relevant Activities. Fort Lewis, WA, February 8-13, 1998
- Effects of Tray Ration Consumption during a 63-Day Marine Field Exercise. Bahamas, 1998
- Food Intake Assessment and Analysis at Fort Drum, NY under the direction of Natick Laboratories, 1998-1999
- Assessment of Energy Expenditure and Nutritional Status of Women Aboard Ship, San Diego, 2000
- Nutritional Intake and Energy Expenditure of Special Forces in Garrison, Colorado Springs, CO, July, 2000
- Assessment of Weight Status and Attrition of Female Marine Recruits during Recruit Training. Parris Island, May, 2001

Reportable Outcomes:

- Development of the Military Dietary Assessment System (MiDAS), a special application designed to enter data from military field trials-modifiable for each trial.
- Internet based access to the Pennington Biomedical Research Center's MENU (Moore's Extended Nutrient) Database for use by military nutrition division dietitians at USARIEM.

Conclusions:

This task provides valuable support for field studies and for the updating and operation of MiDAS. It is a valuable asset for assessment of nutrient intake in soldiers and for cataloguing the nutrient composition of the food supply of relevance to military needs.

6. Task 6: Enhancing Military Diets – Background:

This task was initially under the leadership of Alana Cline, PhD, and later was led by Don Williamson, PhD. The initial purpose of the task under Dr. Cline involved development and incorporation of modified recipes into the Armed Forces Recipe File, instruction of staff and students at the Army Culinary Arts School on selection and preparation of lower fat foods, assess dining hall consumption of menu items, and implement a nutrition education component. In the second year of the grant, the focus shifted to assessing food preferences of military personnel and consumption by military personnel. In year three, Dr. Don Williamson assumed leadership of this task. He designed two studies to evaluate methods for enhancing the consumption of fruits, vegetables, and milk products by soldiers. Soldiers enrolled in Basic Combat Training

(BCT) at Ft. Jackson, SC, were selected for study. When the grant encountered funding difficulties in FY02-03, Dr Williamson submitted and was awarded a DoD grant to continue the work, as is outlined below.

Key Accomplishments under Dr. Cline:

- Dr. Cline's leadership occurred during years 1 and 2 of the grant. During year 1, 38 modified recipes were developed and Dr. Cline's research team traveled to the Army Culinary Arts School at Ft. Lee, VA to coordinate training workshops. Members of the DOD Food Policy Council and Armed Forces Recipe Service held their meeting at PBRC November 1997 to coordinate dissemination of recipe changes among the services
- In year two of the grant, under the direction of the Army Chief Dietitian, OTSG, Dr. Cline modified the task to re-direct our involvement to include analysis of current eating practices of young military members and intervention to improve nutritional status. The Armed Forces Recipe Service indicated that the no longer needed the development of modified recipes, due to changes in the food procurement system.
- Food preference surveys were distributed in dining facilities at 9 military installations to 2818 individuals with a 90% return rate. The gender distribution was 81% male and 60% were 18-21 year age range. All areas of the country were geographically represented. The results of this survey are found in Quarterly and Annual Reports.
- A food consumption survey and acceptability study was conducted at Ft. Lee, VA, Oct 27-30, 1998. The results of this study are found in Quarterly and Annual Reports.

Key Accomplishments under Dr. Williamson:

- From January – March 2000, an observational study of food selections and food intake was conducted on soldiers in BCT. This study has the following key accomplishments:
- Digital photography methodology was developed as a means of measuring food selections and food intake in military dining facilities
- Use of palm held bar code readers was used to unobtrusively track data collection for individual soldiers across eight weeks of BCT
- Soldiers expressed an interest in learning about healthy nutrition, but did not report frequent use of current nutrition education used in BCT
- The selection of fruits by BCT soldiers was below minimal nutritional standards for very active adults
- Fruit selections and fruit intake might be enhanced by replacing "fruit" beverages with 100% fruit drinks
- Milk selections and intake improved significantly over the eight weeks of BCT, changing from below USDA recommended values at week 1 to above recommended values at week 8, on average

- Soldiers consumed adequate amounts of vegetables, on average, but a substantial portion of vegetable intake could be attributed to eating potatoes
- Healthy food selections tended to improve over the course of BCT
- Environmental factors such as time to select foods and placement of foods along the serving line had only modest associations with food selections and food intake
- Soldiers lost an average of about 4 lb. over the eight weeks of BCT
- A new measure of stress during BCT, the Military Stress Scale, was developed and found to be reliable and valid
- Stress, anxiety, and mood improved over the eight weeks of BCT
- Psychological status of the subject, i.e., stress levels, mood, and anxiety, were not significantly associated with food selections or food intake
- Dr. Williamson developed a project in collaboration with USARIEM to address improving military weight and fitness compliance at Fort Bragg. The project was presented to the Committee on Military Nutrition Research in March 2002 and was given approval for development. The initial planning for the project was accomplished under this award and the project received independent funding.
- Dr. Williamson's project "Military Health Behaviors: Promotion of Healthy Weight and Fitness in Career Personnel, DAMD 17-03-2-0030, was awarded \$1million per year for 4 years, beginning in 2003.
- Dr. Williamson's project, "Weight Measurements and Standards for Soldiers," Contract Number W81XWH-05-2-0082, was awarded \$1.7 million for three years. The purpose of this study is to address military weight and fitness compliance for Army reservists assigned to the 94th Regional Readiness Command (RRC).

Reportable Outcomes:

- Dr Ryan and Dr. Williamson organized a Weight Management Skills Training Workshop at the Second Annual DoD Population Health and Health Promotion Conference on 8/11/2002.
- Graduate student working on the project receiving a master's degree: Emily York-Crowe, M.S.
- Graduate student working on the project receiving a doctoral degree: Stephen Mayville, Ph.D.
- Post-doctoral fellow working on the project who was promoted to the PBRC faculty: Robert Newton, Ph.D.
- Digital photography method was used a key measurement tool for the NIH-funded Wise Mind project and for the USDA-funded LA Health project.
- Dr. Don Williamson received funding from 07/1/04 – 07/1/08 under a USDA cooperative agreement with Pennington Biomedical Research Center for the project, "LA Health: A prospective study of primary and secondary obesity prevention in children and adolescents."
- Dr. Don Williamson was funded from 10/01/02 – 09/30/05 by the for NIH 1 R01 DK063453-01, "Wise Mind: Environmental Approach for Obesity Prevention."

The project will test the efficacy of an environmental approach for the prevention of weight gain in children in the second to sixth grades. The efficacy of this prevention program will be compared to an active control group that provides a substance abuse prevention program. This project will test the efficacy of a school-based approach for obesity prevention. The study will include primary and secondary obesity prevention strategies.

Conclusions:

The conclusion from these studies was that food selection practices of young military members are similar to civilian counterparts of the same age. As selections change, nutrition intervention will need to focus on educating service members, dining facility managers and food purchasing agents to assure that the food selections meet performance requirements.

The study of soldiers at Ft. Jackson found that BCT soldiers are interested in learning more about healthy nutrition, but are often not utilizing current nutrition education programs and information. They are selecting diets that are low in fruits and milk products at the beginning of BCT. The consumption of milk products improves during BCT, but fruit intake remains low. Environmental and psychological factors do not appear to be especially good targets for modification of the diets of these soldiers.

7. Task 7: Stress, Nutrition and Immune Function Laboratory – Background:

This task was led by David Horohov, DVM, and the initial thrust of the research was to evaluate stress, nutrition and immune function in mouse models. The work resulted in one publication (Horohov DW, Pourciau SS, Mistic L, Chapman A, Ryan DH. Increased dietary fat prevents sleep deprivation-induced immune suppression in rats. *Comparative Medicine* 51(3):230-233, 2001). Because of the limited productivity of this Task, the Task became inactive in year 3 of the grant.

Key Research Accomplishments:

Two model systems for investigating stress-induced immune modulation were developed:

- A restraint stress model which provides a mild-moderate stress and
- A sleep-deprivation model which caused a more severe stress.
- Sleep deprivation resulted in a loss of body weight and diminished *in vitro* lymphoproliferative response. Sleep deprivation also interfered with the rats' ability to respond to a test vaccine. While control rats produced both humoral and cellular immune responses to the vaccine, the sleep deprived rats failed to respond. It is noteworthy that this effect was present after only 24 hours of sleep deprivation even though *in vitro* responsiveness to mitogen was not affected at this time.

- The restraint model produced a diet-dependent change in the lymphoproliferative response of the rats. Splenocytes from rats on a low fat diet exhibited a minor alteration in their lymphoproliferative response to the mitogen following restraint. By contrast, splenocytes from restrained rats on a high fat diet exhibited a marked alteration in this response. The addition of IL-2 to these cultures failed to overcome this inhibition, even though IL-2R expression was elevated compared to unrestrained controls. These results indicate that the block in the proliferative response could be occurring post-receptor binding, perhaps involving the receptor signaling pathway for IL-2.
- The effect of dietary fatty acid on sleep-deprivation induced immune deviation was assessed in the rats. Immune function was determined both using *in vitro* and *in vivo* measures. Preliminary studies characterized the effect of different dietary fatty acids on immune function. Subsequent studies determined the effect of the diets on sleep-deprivation induced immune modulation. Our results were both consistent with prior reports regarding the effect of dietary fat on baseline immune function and also novel in regards to the immunoprotective nature of some of the diets.
- The possible impact of alternative fat sources on both stress models was also investigated, with emphasis on comparing the effects of omega-3 and omega-6 PUFAs on both baseline immune responses as well as on stress-induced immune modulation in the two model systems.
- These studies demonstrated that sleep deprivation causes a profound alteration of both *in vitro* and *in vivo* immune responses. The altered *in vivo* response included both cellular and humoral immune responses to an alum-adjuvanted vaccine.
- These studies also provide the first evidence that the immunomodulatory effect of sleep deprivation can be offset by increasing the fat content of the diet.

Conclusions:

The impact of stress and sleep deprivation are of importance to the military and there is a need to identify nutritional remedies to the adverse impact of these factors on immune function. Our studies demonstrated that fat content and quality may have an immunomodulatory effect and point to the need for additional studies in animal models.

8. Task 8: Metabolic Unit Project - Background:

In 1993 the PBRC Metabolic Unit was used for a special inpatient study. A description of this experience serves to illustrate how other studies might be developed to address military problems.

The PBRC served as the site for two research cohorts of U.S. Army Rangers. That project was, "Assessment of Intra- and Inter- Individual Metabolic Variation in Special Operations Forces Soldiers." The PI for the project was Ms. T. E. Jones,

affiliated with the Military Nutrition Division at USARIEM. Co-Investigators were C. Gabaree, Lt. Col. T. C. Murphy, Donna Ryan, M.D., and E. Brooks, R.N., M.N.

The purpose of the study was to evaluate a group of Special Operations Forces (SOF) volunteers to determine the metabolic variation during rest, exercise and post-exercise recovery of the individual soldiers. On June 11, 1993, 10 SOF soldiers arrived to serve as the first cohort for testing. Army personnel at the PBRC included Tanya Jones (PI), Sven Ljamo, M.D. (Medical Monitor), Catherine Gabaree (Exercise Physiologist), Lt. Col. Cliff Murphy (Dietitian) and three civilian spotters for exercise testing. The first cohort of military volunteers and civilians left the PBRC on July 1, 1993. There were minimal complications that occurred in the SOF volunteers (subungual hematomas, muscle soreness, and poison ivy dermatitis). All procedures were carried out safely and satisfactorily. A mid-course correction session at the end of the first cohort stay resulted in minor procedure adjustments. From July 9-24, 1993, 10 members of the SOF from the 10th SFG at Fort Devens, Massachusetts participated in the study. All procedures were carried out safely and satisfactorily.

The Metabolic Unit project demonstrated that carbohydrate loading produced increments in physical performance in SOF soldiers. However, the variation between individual soldiers was not great enough to support developing individualized carbohydrate supplements. As a result of this work, the SOF did not pursue a plan to develop individualized soldier supplements for SOF. Therefore, this lack of metabolic variation does not mean that carbohydrate loading would not be effective and the military will pursue carbohydrate loadings for high intensity exercise operations for our SOF soldiers.

The PBRC Metabolic Unit was also used for studies on sleep deprivation (16,17). These included a comparison of tyrosine, against phentermine, caffeine, and d-amphetamine during sleep deprivation with analysis of effects on sleep and on cognitive and motor performance. PBRC has also been a site for a clinical study for the evaluation of minimal or non-invasive methods for field assessment of nutritional and metabolic status. Cpt. Mark Kellogg served as the PI for this project. We have been asked by USARIEM to continue this project's availability for the upcoming grant cycle and have plans to expand our facilities to magnetic resonance spectroscopy studies to accommodate projects in 2006 and beyond.

For much of the grant period, the metabolic unit was inactive as far as Army studies are concerned. At the close of this grant the need for support at the PBRC site for special studies in humans has resurfaced. We are expanding our Metabolic Unit capabilities with a multinuclear magnetic resonance system, to be installed in 2005. The system operates at a strength of 3.0 Tesla and sets a new standard for state-of-the-art in situ biochemistry and imaging. The system contains multiple expandable channels and high bandwidth receivers that ensure unparalleled image reconstructions from all pulse sequences. The multinuclear system allows for the tracing of hydrogen, carbon, and phosphorus atoms within biological molecules such as glucose and fatty acids.

This instrument is critical for the development of research protocols that will determine the optimal nutrients for military performance. Similarly, this instrument can be used to probe the basic biochemistry of the cell, evaluate ergonomic aids, and identify defects in cellular metabolism that are related to peak physical performance. This technology is not currently available within the DoD research environment and will provide state-of-the-art capabilities in military nutrition research. Peak physical performance is required for the elite warfighter. At its core, physical performance is the utilization of nutrients to perform work. Most of our knowledge of the energy producing mitochondria comes from animal studies or ex-vivo studies. This is obviously an artificial situation. The emerging field of magnetic resonance spectroscopy allows us to probe the biochemistry of the cell in vivo in a non-destructive manner, with no radiation and during the performance of work. These kinds of studies are important, because our understanding of how nutrients influence cellular bioenergetics in-vivo is weak.

Key Research Accomplishments:

Since the Metabolic Unit Task was inactive for the period of this grant there are no key accomplishments to report.

Conclusions:

PBRC offers access to the Department of Defense to the finest facilities in the United States for the study of energy balance. PBRC's inpatient and outpatient facilities are unequaled in capability for the study of nutritional issues. This Task can be activated at any time in collaboration with our project officer at USARIEM.

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APPENDIX

FACILITIES DESCRIPTION

A. General Description

- The Pennington Biomedical Research Center (PBRC) is a 530,000 square foot (SF) facility. The initial 245,000 SF facility was completed in 1986 and comprises four separate units (administration building, basic sciences building, clinical unit and comparative medicine). This 40,000 SQ Administration Building includes a 94-seat auditorium and the library. The library has subscriptions to 85 journals, is a branch of the LSU Health Sciences Center Library, and affiliates with the extensive holdings of the Middleton Library on the LSU-Baton Rouge campus. The CD Plus ROM system, based on MEDLINE and FAX facilities, provides rapid access to all relevant scientific literature not available in library holdings.
- The Conference and Education Center complex (95,000 SF) includes the conference center, the residence hall, an exercise facility and a gazebo. This unit houses behavioral faculty, research interventionists and the internet intervention groups.
- The original Laboratory Building has two floors and houses 20 basic science laboratories containing modular work areas designed to adapt laboratory design to individual work habits. Most laboratories have windows overlooking the landscaped patio or adjacent Conference Center. Adjacent to the laboratories are 16 cubicles on each floor (total=32) to house graduate students and post-doctoral fellows. A new connected 190,000 SF laboratory building was completed in November, 2003. It contains two major research floors, each containing four 5,000 SF open laboratories, plus support rooms surrounding a central Core facility. One floor will contain the Genomics Core and the other floor a Neurobiology/Microscopy Core. There are four cubicle areas in this building for postdoctoral fellows. There are warm and cold rooms available in each building. A Mass Spectrometry/Proteomics Core is located in the old building, as is the Clinical Chemistry Core facility.
- The Comparative Medicine Building is housed in a separate wing. The state-of-the-art facility was designed and is supervised by the faculty of the LSU School of Veterinary Medicine, and is a superb animal care facility with specialized laboratory, quarantine, surgery, and behavioral suites. A Transgenic Laboratory is located adjacent to the Comparative Medicine area.
- The two-story Clinical Research Building contains facilities for both outpatient and inpatient research subjects enrolled in clinical trials and is equipped for feeding studies. On the ground floor, 15 outpatient interview and treatment rooms are provided. The 2,600 SF metabolic kitchen and inpatient facilities are located on the second floor. The inpatient unit consists of seven rooms, which can accommodate one or two research subjects in each room, a procedure suite, and two room-size indirect calorimeters. The Clinical Research Facilities are described in more detail below.
- The PBRC has a specially equipped vehicle for use as a mobile metabolic laboratory. The van has space for two SensorMedics metabolic carts and a small

service lab. The PBRC also has a 12-seat passenger van that will be utilized for passenger taxi service or food delivery.

In 1994 the \$20 million Claude B. Pennington, Jr. Nutrition Conference and Education Center was completed. This complex includes the following buildings:

- An office building for the Pennington Foundation
- A 500 seat auditorium with appropriate break-out conference rooms
- Offices to house faculty and staff
- 10 apartments for visiting scientists and a sleep laboratory
- An exercise training and testing facility, with pool, tennis courts and lighted jogging path around a 17 acre lake.

B. Description of Selected Laboratories and Services

1. Mass Spectrometry/ Stable Isotope Core

A Stable Isotope Laboratory has been developed at the PBRC. Stable isotopes, or heavy atoms, are used as tracers to study human metabolism. Since stable isotopes are nonradioactive, they pose no hazard to the patient and can be used in infants, children, and young adults. However, the lack of radioactivity makes detection and quantitation more difficult, necessitating high-technology measuring equipment. We have three modern, magnetic sector, isotope ratio mass spectrometers, as well as automated equipment for sample preparation before introduction into the mass spectrometer. In addition, the stable Isotope Laboratory has two quadrupole mass spectrometers.

The Stable Isotope Laboratory consists of a 2,000 sq. foot basic laboratory for sample preparation. This lab uses a variety of preparation techniques, including electrophoresis, thin layer chromatography, solid phase extraction, vacuum hydrolysis, open column chromatography, and a variety of derivatization techniques. Two vacuum transfer lines are used to micro-distill water from biological fluids into tubes containing zinc. The tubes are closed with a flame and heated in a muffle furnace (500 C) for reduction of water to hydrogen. The tubes are then placed in one of the 12-port automatic tube crackers on the Finnigan MAT 252 for automated isotope ratio measurements.

In addition, the Stable Isotope Laboratory has two 525 sq. foot mass spectrometry laboratories. A Hewlett Packard 5988 quadrupole mass spectrometer is located in one of these labs. This mass spectrometer has a direct insertion probe and GC interface, EI and CI capabilities, and positive or negative ion monitoring, for measurement of any stable isotope labeled (e.g. ^2H , ^{15}N , ^{13}C) organic compounds. The lab also has a high Performance Triple-Stage MS system (Finnigan TSQ 7000) that increases capacity and capability. This instrument provides MS/MS capabilities, to select "parent" ions and scanning fragments, significantly increasing the ability to identify and quantitate compounds of interest. This mass spectrometer has both a GC, as well as an atmospheric pressure ionization and electrospray HPLC interface, EI and CI capabilities, and positive or negative ion monitoring, for measurement of any stable

isotope labeled (e.g. ^2H , ^{15}N , ^{13}C) organic compounds. This instrument also has the capability to determine the molecular weight and sequence of peptides.

Three Finnigan isotope ratio mass spectrometers (a Delta S and 2 MAT 252's) are located in the second mass spectrometry laboratory. An automated CO_2 -water equilibrator is interfaced to one MAT 252 and a new automated device for deuterium preparation is interfaced to the second MAT 252 for large throughput of ^{18}O and ^2H samples for the doubly labeled water energy expenditure method. An automated trapping box for analysis of ^{13}C enrichment of breath CO_2 samples for measurement of substrate oxidation is interfaced to the Delta S. In addition, a GC-combustion unit, for measurement of ^{13}C enrichment of individual peaks eluting from a capillary GC column is interfaced to the Delta S. This instrument is capable of measuring ^{13}C enrichment with a precision of 0.001 atom %, compared to 0.1 atom % capable with the quadrupole instrument. The second MAT 252 has an improved GC-combustion/reduction unit, for measurement of ^{13}C or ^{15}N enrichment of individual peaks eluting from a capillary GC column.

The Stable Isotope laboratory services are utilized in multiple research projects. The Lab is currently overseen by the Associate Director, Jennifer Rood, Ph.D. There are three research associate technicians who complete the procedures to support the research projects using this core facility.

Other resources include a Centrifuge room that houses five Superspeed Beckman and Sorval centrifuges with multiple rotors.

2. Clinical Research Laboratory

The Clinical Research Laboratory is directed by Jennifer Rood, Ph.D., DABCC, FACB. Dr. Rood has initiated rigid quality control systems in the lab and the laboratory is accredited by the Health Care Financing Administration (HCFA) and the College of American Pathologists (CAP). The laboratory also participates in the lipid standardization program of the Centers for Disease Control. The Good Laboratory Practices guidelines are being followed in the laboratory. The Clinical Research Laboratory at Pennington Biomedical Research Center performs analyses for PBRC clinical trials, for basic researchers at the Center, for the US Army Institute of Environmental Medicine (USARIEM), and for contracting clients. These relationships have also provided experience in cooperative, efficient and accurate exchange of analytical information with other scientists. The Clinical Research Laboratory is staffed by licensed medical technologists, phlebotomists, and accessioners.

The laboratory is well-equipped for performing routine and specialized tests on clinical subjects. In 2004 over 250 analytical procedures, involving 200,000 tests and 7,500 venipunctures were performed by the lab.

The following instrumentation and assays are available:

Agilent Technologies HPLC

Alpha tocopherol	Lutein/Zeaxanthin
Amino Acids	Lycopene
Beta Carotene	Retinol
Bromide	

Antek 9000

Nitrogen, urine, sweat and fecal

Bayer Clinitek 200

Urine blood	Urine pH
Urine glucose	Urine protein
Urine ketones	Urine specific gravity
Urine leukocyte esterase	Urine urobilinogen
Urine Nitrite	

Beckman Coulter Array

Apolipoprotein A1	IgM
Apolipoprotein B	Lp(a)
Ceruloplasmin	Prealbumin
Haptoglobin	Retinol Binding Protein
Ig A	Transferrin
Ig G	

Beckman Coulter Hmx

Complete Blood Count	Platelet Count
Red blood cell count	Mean Platelet Volume
Hemoglobin	White Blood Cell Count
Hematocrit	Basophil # and %
MCV	Eosinophil # and %
MCH	Lymphocyte # and %
MCHC	Monocyte # and %
Red cell distribution width	Neutrophil # and %

Beckman Coulter Synchron CX5

BHBA	Glycerol
Caffeine	Lactate
FRAP	Lipase
Free fatty acids	Vitamin C
Fructosamine	

Beckman Coulter Synchron CX7

Albumin	HDL cholesterol
Alkaline phosphatase	Hemoglobin A1C
ALT	Iron
Amylase	Lactate dehydrogenase

AST	Magnesium
Blood urea nitrogen	Phosphorus
Calcium	Potassium
Carbon dioxide	Sodium
Chloride	Total bilirubin
Cholesterol	Total Iron Binding Capacity
Creatine kinase	Total Protein
Creatinine	Triglycerides
Direct bilirubin	Urea
GGT	Uric Acid

ELISA – P Lab or Bio Rad Plate Reader

IGF1	Blood contamination, saliva
IGF2	Cortisol, saliva
IFGBP1	DHEA, saliva
IGFBP3	Progesterone, saliva
PAI-1	Testosterone, saliva
TNF α	Vitamin B6
Protein carbonyls	Asymmetric dimethyl arginine

Bio Rad HPLC

Epinephrine	Norepinephrine
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Calculation

% Saturation	Absolute granulocytes
LDL Cholesterol	Osmolality

Diagnostic Products Corporation 2000

C Peptide	FSH
C Reactive Protein	Growth Hormone
Cortisol	Homocysteine
DHEA-S	Insulin
Estradiol	LH
Free T3	Progesterone
Free T4	Prolactin
PTH, intact	T3
SHBG	T4
T Uptake	TSH
ACTH	Folate
Cotinine	Myoglobin
Deoxypyridinoline	PSA
Ferritin	Vitamin B12

Helena Laboratories Hematofluorometer

Zinc protoporphyrin

IL ACL 3000+

Factor VII
Fibrinogen

Prothrombin Time

Luminex Labmap 100

Cytokines (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, GM-CSF, IFN- γ , TNF- α)

Manual Procedures

Urine Appearance
Urine Color

HCG
Occult Blood

Microscopic analyses (urine)

Amorphous	Epithelial cells
Bacteria	Mucus
Casts	Red blood cells
Crystals	White blood cells

Packard Riastar gamma counter

1,25 dihydroxy vitamin D	Free testosterone
17 hydroxy progesterone	Glucagon
25 hydroxy vitamin D	Leptin
Adiponectin	Neuropeptide Y
Androstenedione	Osteocalcin
Bone specific Alkaline Phosphatase	Reverse T3
DHEA	Testosterone
Estrone Sulfate	Ghrelin
Angiotensin II	Renin
PYY 3-36	

Perkin Elmer AA Spectrophotometer

Cadmium

Perkin Elmer LS50B luminometer

ORAC

3. Animal Facilities

The Comparative Biology Building is housed in a separate wing. It is an AAALAC-accredited facility that encompasses 40,000 SF of state-of-the-art animal housing, laboratories, and support. The animal facility is designed with a clean and dirty corridor system. The dirty side of the cage washing room is located in corridor (E1025). There are 30 animal rooms. Fourteen of the animal rooms are committed to mouse breeding. The main strains of mice bred are C57BL/6, SWR/J, AKR/J, and FVB/NJ. Many of our strains are used in production for transgenic, congenic, or coisogenic

research. Five of the animal rooms are committed to rat breeding. All rooms have the capacity for automated watering of cages. There are seven Bioclean units for housing mice or rats. Support area includes three shared laboratories that are approximately 400 SF in size. These rooms are equipped with built in hoods, cabinets and sinks. The one necropsy room is equipped with a laminar flow necropsy table, AMSCO Eagle 2021 sterilizer, cabinets, chest freezer, surgical lighting, and a vivistat.

A recent expansion had added additional space that is barrier-protected and includes: a) a new suite for breeding animals; this area includes both a procedure and staging area; b) a new transgenic laboratory with seven adjacent animal rooms; c) seven additional animal rooms for other breeding programs; d) additional storage area for bedding, food, and equipment. All of these facilities are in a clean barrier-protected area. In addition, there is a new larger quarantine area with three animal rooms and one procedure room with two adjoining quarantine rooms. Renovation of the cage washing area, with the addition of a Girton Bedding Dispenser that can fill 24 cages per minute or 1440 cages per hour, is underway.

The facility currently handles approximately 10-11000/day. There are currently 15 rooms of varying sizes that are allocated for behavioral studies other than feeding. The equipment housed in these rooms is described below in the Behavioral Phenotyping Core.

4. Description of the Clinical Research Facilities in the PBRC

The two-story Clinical Research Building houses a 16,000 SF outpatient clinic and a 20,000 SF inpatient unit. This facility is dedicated to the clinical research trials for the PBRC. The unit is designed for easy access and convenience of research volunteers. The PBRC's central location in Baton Rouge, covered drive-up entry and spacious free parking are assets encouraging research subject participation. Accommodations for on-site dining and convenient take-out food service and a food delivery service facilitate feeding studies. The activity of the Clinic over the years 1992-2003 is summarized below in Table A. The ground floor includes 10 general examination rooms and five interview rooms which are utilized for outpatient studies. One exam room is adapted for phlebotomy use. Another room is equipped for determination of height, weight and anthropometrics. There is a classroom which can accommodate 50 persons for educational sessions. The unit also contains administrative office space, reception and waiting areas, a medical records library, a pharmacy, and recruiting offices. The 3,000 SF clinic annex contains 16 private offices and two conference rooms that can accommodate up to 50 people.

The second floor contains the following:

1. Metabolic-kitchen (described in more detail below)
2. Two indirect room-size calorimeters for the Metabolic Chamber Group (described in more detail below)
3. Two rooms for indirect calorimetry (eight stations total) and DEXA instrumentation

4. Inpatient dining area
5. Seven inpatient rooms (one to two beds/room)
6. Nurses' station
7. Satellite pharmacy
8. Procedure room (eight chairs) for FSIGTT, oral glucose tolerance testing and meal tests
9. Ultra sound suite for cardiac echo and peripheral vascular studies
10. Biopsy room for skeletal muscle and adipose biopsy
11. Adipose tissue microdialysis analysis
12. Heart rate (Heart rate) variability

Table A. Clinic Activity Report

YEAR	TELEPHONE CONTACTS	# SCREENING VISITS	# ENROLLED IN STUDIES	STUDIES STARTED
1991				3
1992	1962	771	255	5
1993	2543	1124	575	14
1994	3742	1423	451	15
1995	6020	2005	574	7
1996	4799	1432	628	13
1997	4261	1667	612	11
1998	5282	2218	783	25
1999	4537	1686	618	13
2000	5458	1933	731	24
2001	4432	1707	804	26
2002	4388	1836	999	24
2003	6234	2392	1373	29
TOTAL	53,658	20,194	8403	209

Metabolic Kitchen and Food Preparation

The PBRC metabolic kitchen is located on the second floor of the Clinical Research Unit and has 2,622 SF of working space. The kitchen area is divided into four fully-equipped individual kitchen areas, each comprising 130 SF. These individual kitchens are ideal for conducting simultaneously various protocols. Each individual kitchen area is equipped with a refrigerator, freezer, microwave, cook-top, five-quart mixer, one-quart blender, toaster, and electronic balances. One kitchen area is set up as a bake area, containing a 20-quart mixer. The 440 SF large quantity food preparation area contains state of the art convection ovens, steam ovens and kettle, bake ovens and cook-tops, microwave, food warmers, food chopper, slicer, food processor, one-gallon blender, and a large capacity electronic balance. There is also a tray service area; dish room; and approximately 800 SF for receiving and for dry, refrigerated and frozen storage in the storeroom, walk-in refrigerator, and walk-in freezer. An additional food storage area with space for dry storage, refrigeration and

freezer storage is located adjacent to the service road. Approximately 225 meals per day can be prepared in the facility. Study participants consume their meals in the 850 SF dining area, and meals may be provided for take-out.

The PBRC metabolic kitchen is under the direction and supervision of Marlene M. Most, Ph.D., RD, LDN, FADA. The kitchen is managed by Rachel Romaine. The Metabolic Kitchen is staffed with research dietitians, research associates, food service workers/cooks, a stock clerk, student workers, and part-time hostesses. The research dietitians have the primary responsibility for planning and managing the dietary component of individual feeding study protocols. The research associates, cooks, and food service workers prepare and serve the research designated diets.

Menus are developed by the metabolic kitchen research dietitians. Specified food products, if needed, are developed by the metabolic kitchen staff. Recipes are selected to include regional food preferences to increase dietary adherence. After taste-testing, the food products are analyzed for nutrient content, and then included in the database for menu planning. The research dietitian reviews the finalized menu with each potential participant before the research project begins. The participant's food likes, dislikes and intolerances, including food allergies, are discussed. Food purchases are based on specifications outlined during the menu development to meet nutrient content requirements. At receiving, the product is inspected by the stock clerk to ensure proper quality and weight specifications. When possible, foods to be used throughout the research study are purchased at one time from a single lot to ensure minimum variation, and are stored properly. Bulk food deliveries are stored in adjacent cold, dry or freezer space. Standardized recipes outlining specific ingredients and gram weights, correct mixing and cooking procedures, timing, and use of equipment are meticulously followed under sanitary procedures. All ingredients are weighted to 0.1 gram on electronic balances. Mixed foods are prepared in batch quantities, large enough for the entire study. Those foods then are individually portioned, weighed, sealed, labeled, and frozen until ready to use. The nutrient composition of every study diet may be verified by chemical analysis of aliquots of each menu cycle to ensure that the designed menus achieve the target nutrient values predicted by the nutrient database. A continuous quality assurance program is followed to check food item weights, recipe procedures, packed meal and tray assembly, and food temperatures. Documentation is maintained for each study. Furthermore, operational problems are documented with an appropriate plan of action and follow-up. All food service employees receive training in food sanitation and in research diet preparation.

Daily food production sheets for each participant are used when preparing the meals, listing day, menu cycle, food items required with portion weights, and special dietary requirements. Foods are labeled for participant identification. Foods are placed on individual meal trays until served, or are individually packaged for take-out, following tray assembly forms. At the time of meal pick-up the hostess reviews the menu with the participant to confirm all foods are provided. Meals are served to the participant on test days only after all study procedures have been completed. A meal ticket is given to the participant by an authorized nurse or scientist, which is presented to the hostess.

Additionally, dietetic research associates obtain daily comments from each participant, and record dietary progress notes. Potential problems with meal acceptance are identified and resolved. Personal attention and encouragement to continue on the diets are provided by all staff members throughout the study.

Food Chemistry Laboratory

The Food Chemistry laboratory at PBRC performs analysis of food for a variety of substances and nutrients. In addition to routine analysis of fat, carbohydrate, protein, ash, moisture and dietary fiber, the lab also measures vitamins, minerals, and other important nutrients.

Equipment in the laboratory includes the following:

- a) Soxtec automated fat extraction system
- b) Fibertec automated dietary fiber system
- c) CEM microwave for automate moisture determination
- d) CEM microwave for automated ash determination
- e) Two Hewlett Packard gas chromatographs for total fat, fatty acids, and cholesterol
- f) Two Water HPLC systems for vitamins and carbohydrates
- g) Hewlett Packard capillary electrophoresis system for charged ions and vitamins
- h) Assorted food processing equipment (blenders, sonicator, homogenizer, food processors, etc.)
- i) Assorted laboratory equipment (incubators, water baths, computers, etc.)

The laboratory serves as a support laboratory for the MENU database and the metabolic kitchen. The laboratory is instrumental in the design, verification, and maintenance of specific diets which are designed using the database and prepared in the metabolic kitchen.

Clinical Metabolism Laboratory

The energy expenditure laboratory consists of two Respiration chambers (whole-room indirect calorimeters) and eight portable ventilated hood systems (Datex-Ohmeda). The respiration chambers are located in the inpatient area of the Clinical building. The two chambers are each 14'5" x 10'7" with 8' ceilings corresponding to a total volume of about 27000 L. Each chamber is provided with furnishings and equipment necessary to perform metabolic studies on research volunteers over extended time periods in a precisely controlled environment. The chambers are large enough for individuals to live for periods up to one week. They are equipped with a bed, chair, desk, toilet, sink, refrigerator, TV, VCR, computer with internet access, and motion sensors. For both the respiration chambers and the ventilated hood systems room air is drawn through the chamber at a known flow rate, and oxygen and carbon dioxide concentrations are measured in in- and outgoing air are measured for the calculation of oxygen consumption and carbon dioxide production from which energy expenditure is calculated. If nitrogen excretion is also measured, substrate oxidation rate can be calculated as well. The laboratory is overseen by Lilian de Jonge, PhD (*in*

the directory the lab is only in my name). In addition, the unit employs a fulltime engineer to ensure accurate data collection and to perform on-going maintenance and calibration of the equipment. Two research associates assist in the performance of the tests and provide care to the volunteers.

Body Composition Laboratory

The center is well equipped to do sophisticated analyses of body composition and relate determinations. Techniques available include dual X-ray absorptiometry (DEXA, Hologics QR 2000), densitometry (under water weighing), deuterium dilution, bio-electrical impedance assessment (BIA), and anthropometry. The center routinely conducts body composition assessment on about 200 participants each month, supporting all research programs on humans and many animal projects as well. Contracts for computed tomography (CT scan) and magnetic resonance imaging (MRI) measurements are available through collaborations with several area hospitals and diagnostic clinics. As of January 1, 1996, we have installed on-site a computer server/analysis system, which permits direct electronic importing of CT and MRI scans taken at other locations to the center for editing, storage, and analysis.

All participants in PBRC research projects receive a general screening, which includes DEXA, BIA, and anthropometric determinations. The results of these procedures are entered into an accumulating data base which will eventually permit the establishment of local norms for fatness, muscularity, bone mineral content, and growth and development. In addition, these data are available for retrospective analyses relating body composition to other bio-medical, demographic, or psycho-social characteristics.

The center currently has two certified DEXA technicians. These individuals also conduct BIA and densitometry procedures. In addition, the clinical nursing staff is regularly certified in anthropometric and BIA techniques. As a result, the center has considerable capacity to screen large numbers of research volunteers. Moreover, a mobile research unit, a converted recreational vehicle 35 feet long, weighing 16,000 lbs., is available for field screenings. It is outfitted to accomplish complete anthropometric and BIA assessments as well as perform related energy expenditure and physiological determinations at other locations. This affords great flexibility in contacting target populations outside the metropolitan area or screening local groups with transportation or logistic impediments.

Endocrinology and Metabolism Laboratory

The Endocrinology and Metabolism Laboratory is designed to study the endocrinologic, molecular, physiologic, and dietary variables that related dietary fat intake to the development of obesity and high risk patterns of regional fat distribution. The Laboratory is under the direction of Steven R. Smith, MD. The main components of this laboratory are:

1. Molecular and Physiology Component.

The molecular physiology component of the Endocrinology and Metabolism Laboratory is involved in sampling, preparation and analysis of human fat and muscle samples collected from participants in clinical protocols. The following procedures are utilized by the molecular physiology component of the Endocrinology and Metabolism Laboratory:

- a) muscle biopsy
- b) subcutaneous fat aspiration/biopsy
- c) muscle adipocyte RNA and protein extraction
- d) Western immuno blotting
- e) Northern analysis
- f) RNase protection assays

2. Fatty Acid Analysis

The fatty acid analysis component is designed to utilize fatty acids obtained from serum, erythrocytes, or fat biopsies to directly determine dietary fat intake over the previous two-three days, six-eight weeks and two-three years respectively. This methodology utilized the Hewlett Packard automated gas Chromatograph and began operations on July 1, 1996 with one full time research associate.

3. Medical Imaging Analysis

The medical image analysis system consists a CT/MRI image analysis module, which began operation January 1, 1996. The purpose of this process is to precisely quantitate visceral abdominal and subcutaneous fat mass in 2D or 3D arrays. The hardware consists of:

- Sun Sparc 20 image analysis computer
 - Encryption and password protection for data security
 - Both 4mm DAT tape and 1.3 Gigabyte optical disk backup and storage system
- image visualization of unimodal and multimodal CT/MRI images transferred in a 100% digital modality
- An Internet connection allows for analysis of CT/MRI images obtained at remote sites and therefore remote collaborations as a core service. One research associate is responsible for the retrieval, backup and analysis of scans obtained under the direction of Steven R. Smith, M.D.

Nutrient Database Laboratory

The Nutrient Database Laboratory has two main components. One component, the MENu Database, is overseen by Catherine Champagne, Ph.D. and the other, the chemistry function is overseen by a food chemist. Four research associate technicians support the database function. The data base and chemical analysis components of this laboratory function in a complementary way to serve the research projects in this

proposal. The MENU database was donated to the Pennington Biomedical Research Foundation in October, 1992, by its owner and developer, Dr. Margaret C. Moore. The Extended Table of Nutrient Values has been renamed to honor the name of its developer. The Moore Extended Nutrient Database, now known as the MENU Database, is an appropriate reflection of its current use in analyzing menus and recipes for the PBRC metabolic kitchen, for school lunches in Louisiana, and for multicenter feeding trials.

The MENU Database was selected for use in the National Heart, Lung and Blood Institute multi-center study of diet and lipoproteins. When compared to analytical laboratory values obtained from an outside Food Composition Laboratory, the MENU Database was closer to actual values than the three other databases used to calculate the same menus. Current data from additional menus analyzed still indicated good agreement between values from the MENU Database and the laboratory assays.